

What is claimed is:

1. A Complex, comprising:
 - a. a first nucleic acid comprising, from 3' to 5': a Substrate Hybridization Domain and a Signal Template Domain, wherein:
 - i. the Substrate Hybridization Domain comprises a sequence of about 5 to about 20 nucleotides; and
 - ii. the Signal Template Domain comprises a sequence of about 5 to about 100 nucleotides;
 - and:
 - b. a second nucleic acid comprising from 3' to 5': a Signal Domain, a Template Hybridization Domain and a Target Binding Domain, wherein:
 - i. the Signal Domain comprises a sequence of about 5 to about 100 nucleotides, which sequence shows complementarity toward and is hybridizable to the Signal Template Domain of the first nucleic acid, and of which at least two nucleotides are detectably labeled;
 - ii. the Template Hybridization Domain comprises a sequence of about 5 to about 20 nucleotides, is not detectably labeled, and shows complementarity toward and is hybridizable to the Substrate Hybridization Domain of the first nucleic acid;
 - iii. the Target Binding Domain is not detectably labeled and comprises a nucleotide sequence heterologous to that of the Template Hybridization Domain.
2. The Complex of claim 1, wherein the nucleotides which comprise the first or second nucleic acid are deoxyribonucleotides.
3. The Complex of claim 1, wherein the nucleotides which comprise the Signal Domain of the second nucleic acid are deoxyribonucleotides and the nucleotides which comprise the Template Hybridization Domain and the Target Binding Domain of the second nucleic acid are ribonucleotides.
4. The Complex of claim 1, wherein the Substrate Hybridization Domain is at the 3' end of the first nucleic acid.

5. The Complex of claim 1, wherein the Substrate Hybridization Domain comprises a sequence of about 5 to about 10 nucleotides.

5 6. The Complex of claim 1, wherein the Substrate Hybridization Domain cannot be extended by a 5'→3' DNA polymerase.

7. The Complex of claim 6, wherein the Substrate Hybridization Domain further comprises an extension of nucleotides at the 3' end of said Substrate Hybridization Domain, the extension having no complementarity to the Template Hybridization Domain of the second nucleic acid.

8. The Complex of claim 6, wherein the Substrate Hybridization Domain comprises a 3'-terminal modified nucleotide.

15 9. The Complex of claim 8, wherein the modification is selected from the group consisting of: a 3'-amino-modifier, a 2', 3'-dideoxynucleotide, a 3'-phosphate, and a modified 3'-phosphate group.

20 10. The Complex of claim 1, wherein the Substrate Hybridization Domain comprises at least one nucleotide which comprises a modified cytidine, which nucleotide is selected from the group consisting of: C5-methyl-dC and C5-propynyl-dC.

11. The Complex of claim 1, wherein the Signal Domain comprises a sequence of about 10 to about 50 nucleotides.

12. The Complex of claim 1, wherein the Signal Domain is at least 50%, at least 70%, at least 90% or 100% homopolymeric.

30 13. The Complex of claim 1, wherein at least 60%, at least 80% or 100% of the nucleotides of the Template Hybridization Domain comprise guanosine or cytidine or a combination thereof.

14. The Complex of claim 1, wherein at least 60% of the nucleotides of the Template Hybridization Domain comprise guanosine or cytidine or a combination thereof, and the Signal Domain is at least 50% homopolymeric.

15. A reaction mixture for use in a process for the labeling of a nucleic acid molecule comprising:

- a. a first nucleic acid comprising, from 3' to 5': a Substrate Hybridization Domain and a Signal Template Domain, wherein:
 - i. the Substrate Hybridization Domain comprises a sequence of about 5 to about 20 nucleotides; and
 - ii. the Signal Template Domain comprises a sequence of about 5 to about 100 nucleotides;
- and:
- b. a second nucleic acid comprising from 3' to 5': a Template Hybridization Domain and a Target Binding Domain, wherein:
 - i. the Template Hybridization Domain comprises a sequence of about 5 to about 20 nucleotides, is not detectably labeled, and shows complementarity toward and is hybridizable to the Substrate Hybridization Domain of the first nucleic acid;
 - ii. the Target Binding Domain is not detectably labeled and comprises a nucleotide sequence heterologous to that of the Template Hybridization Domain.

16. The reaction mixture of claim 15, wherein the nucleotides which comprise the first or second nucleic acid are deoxyribonucleotides.

17. The reaction mixture of claim 15, wherein the nucleotides which comprise the first or second nucleic acid are ribonucleotides.

18. The reaction mixture of claim 15, wherein the Substrate Hybridization Domain is at the 3' end of the first nucleic acid.

19. The reaction mixture of claim 15, wherein the Substrate Hybridization Domain comprises a sequence of about 5 to about 10 nucleotides.

20. The reaction mixture of claim 15, wherein the Substrate Hybridization Domain cannot be extended by a 5'→3' DNA polymerase.

21. The reaction mixture of claim 20, wherein the Substrate Hybridization

Domain further comprises an extension of nucleotides at the 3' end of said Substrate Hybridization Domain, the extension having no complementarity to the Template Hybridization Domain of the second nucleic acid.

5 22. The reaction mixture of claim 20, wherein the Substrate Hybridization Domain comprises a 3'-terminal modified nucleotide.

23. The reaction mixture of claim 22, wherein the modification is selected from the group consisting of: a 3'-amino-modifier, a 2', 3'-dideoxynucleotide, a 3'-phosphate, and
10 a modified 3'-phosphate group.

24. The reaction mixture of claim 15, wherein the Substrate Hybridization Domain comprises at least one nucleotide which comprises a modified cytidine, which nucleotide is selected from the group consisting of: C5-methyl-dC and C5-propynyl-dC.

15 25. The reaction mixture of claim 15, wherein the Signal Template Domain comprises a sequence of about 10 to about 50 nucleotides.

26. The reaction mixture of claim 15, wherein the Signal Domain is at least 50%,
20 at least 70%, at least 90% or 100% homopolymeric.

27. The reaction mixture of claim 15, wherein at least 60%, at least 80% or 100% of the nucleotides of the Template Hybridization Domain comprise guanosine or cytidine or a combination thereof.

25 28. The reaction mixture of claim 15, wherein at least 60% of the nucleotides of the Template Hybridization Domain comprise guanosine or cytidine or a combination thereof, and the Signal Domain is at least 50% homopolymeric.

30 29. A method of labeling a nucleic acid molecule, comprising the steps of:

- Subcl
- 35 a. Hybridizing a first nucleic acid to a second nucleic acid, wherein the first nucleic acid comprises, from 3' to 5': a Substrate Hybridization Domain and a Signal Template Domain, wherein:
- i. the Substrate Hybridization Domain comprises a sequence of about 5 to about 20 nucleotides; and
 - ii. the Signal Template Domain comprises a sequence of about 5

to about 100 nucleotides;

and the second nucleic acid comprises from 3' to 5': a Template Hybridization Domain and a Target Binding Domain, wherein:

- i. the Template Hybridization Domain comprises a sequence of about 5 to about 20 nucleotides, is not detectably labeled, and shows complementarity toward and is hybridizable to the Substrate Hybridization Domain of the first nucleic acid;
- ii. the Target Binding Domain is not detectably labeled and comprises a nucleotide sequence heterologous to that of the Template Hybridization Domain;

and:

- b. extending the second nucleic acid with a DNA polymerase in the presence of a labeled nucleotide to create a Signal Domain having a sequence which shows complementarity toward and is hybridizable to the Signal Template Domain, thereby labeling said second nucleic acid molecule.

30. The method of claim 29, wherein the nucleotides which comprise the first or second nucleic acid are deoxyribonucleotides.

31. The method of claim 29, wherein the nucleotides which comprise the first or second nucleic acid are ribonucleotides.

32. The method of claim 29, wherein the second nucleic acid consists of about 15 to about 150 nucleotides.

33. The method of claim 29, wherein the Substrate Hybridization Domain is at the 3' end of the first nucleic acid.

34. The method of claim 29, wherein the Substrate Hybridization Domain comprises a sequence of about 5 to about 10 nucleotides.

35. The method of claim 29, wherein the Substrate Hybridization Domain cannot be extended by a 5'→3' DNA polymerase.

36. The method of claim 35, wherein the Substrate Hybridization Domain

further comprises an extension of nucleotides at the 3' end of said Substrate Hybridization Domain, the extension having no complementarity to the Template Hybridization Domain of the second nucleic acid.

5 37. The method of claim 35, wherein the Substrate Hybridization Domain comprises a 3'-terminal modified nucleotide.

 38. The method of claim 37, wherein the modification is selected from the group consisting of: a 3'-amino-modifier, a 2', 3'-dideoxynucleotide, a 3'-phosphate, and a
10 modified 3'-phosphate group.

 39. The method of claim 29, wherein the Substrate Hybridization Domain comprises at least one one nucleotide which comprises a modified cytidine, which nucleotide is selected from the group consisting of: C5-methyl-dC and C5-propynyl-dC.

15 40. The method of claim 29, wherein the Signal Template Domain comprises a sequence of about 10 to about 50 nucleotides.

 41. The method of claim 29, wherein the Signal Domain is at least 50%, at least
20 70%, at least 90% or 100% homopolymeric.

 42. The method of claim 29, wherein at least 60%, at least 80% or 100% of the nucleotides of the Template Hybridization Domain comprise guanosine or cytidine or a combination thereof.

25 43. The method of claim 29, wherein at least 60% of the nucleotides of the Template Hybridization Domain comprise guanosine or cytidine or a combination thereof, and the Signal Domain is at least 50% homopolymeric.

30 44. The method of claim 29, wherein the extending step is carried out by a DNA polymerase selected from the group consisting of: *E. coli* DNA polymerase I holoenzyme, Klenow fragment of *E. coli* DNA polymerase I, T4 DNA polymerase, T7 DNA polymerase, and a DNA polymerase encoded by a thermophilic bacterium.

35 45. The method of claim 29, wherein the Template Hybridization Domain or the Substrate Hybridization Domain comprises at least one modified nucleotide, which

modified nucleotide increases the hybridization affinity of said Template Hybridization Domain to said Substrate Hybridization Domain.

46. The method of claim 45, wherein at least one modified nucleotide is found in
5 the Template Hybridization Domain.

47. The method of claim 46, wherein at least one modified nucleotide is selected from the group consisting of: C5-methyl-dC, C5-propynyl-dC, C5-propynyl-dU, and 2, 6-diaminopurine.

10 48. The method of claim 29, wherein at least one nucleotide comprises a label selected from the group consisting of: ^{32}P , ^{33}P , ^{35}S , fluorescein, digoxigenin, biotin, Cy5, Cy3, and rhodamine.

15 49. A method for detecting a Target Nucleic Acid in a sample, comprising:
a. contacting the sample with the Complex of claim 1 under conditions whereby said Complex can bind to the Target Nucleic Acid to form a Complex-Target Nucleic Acid hybrid; and
b. detecting any Complex-Target Nucleic Acid hybrids, so that if a Complex-Target Nucleic Acid hybrid is detected, a Target Nucleic Acid is detected in the sample.
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25 50. A method for detecting a Target Nucleic Acid in a sample, comprising:
a. dissociating the Complex of claim 1 to generate a single stranded first nucleic acid and a single stranded second nucleic acid;
b. contacting the sample with the second nucleic acid of step a. under conditions whereby said second nucleic acid can bind to the Target Nucleic Acid to form a second nucleic acid-Target Nucleic Acid hybrid; and
c. detecting any second nucleic acid-Target Nucleic Acid hybrids, so that if a second nucleic acid-Target Nucleic Acid hybrid is detected, a Target Nucleic Acid is detected in the sample.
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35 51. A kit for labeling a nucleic acid molecule, comprising the reaction mixture of claim 15, and a DNA polymerase

52. The kit of claim 51, wherein at least 60% of the nucleotides of the Template Hybridization Domain comprise guanosine or cytidine or a combination thereof, and the Signal Domain is at least 50% homopolymeric.

5 53. The kit of claim 51, wherein the Substrate Hybridization Domain comprises a predetermined sequence comprising CCCGCC and the Signal Template Domain comprises a predetermined sequence comprising TTTTTTTTTT.

54. The kit of claim 51, wherein, the first nucleic acid comprises a
10 predetermined sequence comprising SEQ ID NO:10.

15  The handwritten notes consist of two phrases: 'add B3' and 'Add C4'. 'add B3' is written on the left, with a diagonal line through it. 'Add C4' is written on the right, with a large triangle drawn around it. Arrows point from the text towards the triangle.

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